



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

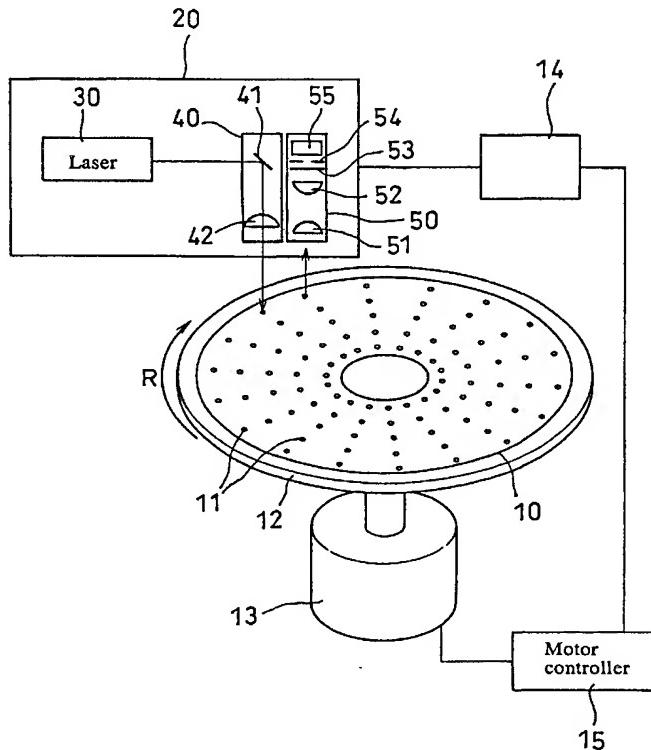
(51) International Patent Classification 7 : <b>G01N 21/64, 33/487</b>	A1	(11) International Publication Number: <b>WO 00/68668</b> (43) International Publication Date: 16 November 2000 (16.11.00)
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(21) International Application Number: <b>PCT/JP00/02996</b>	(81) Designated States: US, European patent (DE, FR, GB).
(22) International Filing Date: 10 May 2000 (10.05.00)	
(30) Priority Data: 11/129365 11 May 1999 (11.05.99) JP	<b>Published</b> <i>With international search report.</i>
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## (54) Title: METHOD AND DEVICE FOR FLUORESCENCE MEASUREMENT

## (57) Abstract

The invention uses a continuous-wave laser as an excitation light source to achieve highly-accurate detection with a simple structure. A sample (11a) is irradiated with excitation light from an excitation light source (30), and then transported to an optical axis of a fluorescence detecting means (50), thereby being detected of its fluorescence intensity.



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## DESCRIPTION

**METHOD AND DEVICE FOR FLUORESCENCE MEASUREMENT****Technical Field**

The present invention relates to a method and a device for fluorescence measurement. More particularly, the present invention relates to a method and a device for fluorescence measurement which is favored for reading a biochip on which fluorescence-labeled samples such as DNAs or proteins are arranged in a plane.

**Background Art**

In the fields of molecular biology and biochemistry, hybridization reaction between a nucleic acid or a protein having a known sequence and a target molecule contained in a sample is employed for searching useful genes or for diagnosing diseases. For this purpose, a biochip is used which has a plurality of sample spots on its surface for processing a mass number of samples in a short time. Each sample spot on the biochip has a different probe immobilized thereon. This biochip is placed into a reaction vessel together with a sample DNA to hybridize probes bound to the sample spots on the biochip with the fluorescence-labeled sample DNA. Then, the biochip is irradiated with excitation light to measure the fluorescent intensity of each sample

spot with a fluorescence measurement device. Accordingly, a binding level between each probe and the sample DNA is obtained, which is then converted into a desired piece of information.

Figure 5 is a schematic view showing a conventional fluorescence measurement device used for reading a biochip. The fluorescence measurement device radiates excitation light to each sample spot on the biochip and incorporates fluorescence emitted from each sample spot via an optical fiber bundle according to a luminous point scanning system.

The surface of the biochip 100 made of a slide glass or the like, has fluorescence-labeled sample spots 101 such as DNAs and proteins in a lattice arrangement. For example, microscopic spots with diameters of 50  $\mu\text{m}$  are arranged placing a space of about 100  $\mu\text{m}$  therebetween in a direction represented by an arrow X. A chip-transporting motor 103 transports the biochip 100 in the parallel direction represented by the arrow Y. A laser beam 107 generated by a laser 105 reflects off a rotary mirror 109 and is guided to the surface of the biochip as a luminous point. The rotary mirror 109 is rotated by a motor 111 in a direction represented by an arrow R. The laser beam 107 linearly scans the surface of the biochip 100 in a direction represented by an arrow X. A motor controller 119 controls the chip-transporting motor 103 and the motor 111 as described above to run the laser beam in X-direction while continuously

transporting the biochip in Y-direction to radiate the whole sample surface of the biochip 100.

The fluorescence emitted from each sample spot on the biochip is guided to photomultiplier tubes (PMTs) 115a and 115b via optical fiber bundles 113a and 113b, respectively. The incident ends of the optical fiber bundles 113a and 113b are arranged in lines corresponding to the scanning line of the laser beam on the surface of the biochip 100. The other ends of the optical fiber bundles 113a and 113b extend to the PMTs 115a and 115b, respectively. Optical filters 117a and 117b are provided between the optical fiber bundles 113a and 113b and the PMTs 115a and 115b, respectively, so that only the fluorescent wavelength of interest is read by the PMTs 115a and 115b. The output from the PMT 115a and 115b is sent to a data processor 121 for data processing. In this manner, a plurality of light receiving systems with optical filters of different wavelength transmitting ranges are provided to allow polychromatic reading.

The light receiving systems of the above-described conventional fluorescence measurement device receive not only the fluorescence from the sample but also excitation laser beam reflected or scattered off the sample surface. Since the amount of the sample is microscopic, the quantity of excitation light incident to the light receiving system is so many times greater than the quantity of the fluorescence incident to the same. Thus, the optical filter needs to have

a narrow wavelength transmitting range to eliminate the excitation light, in which case the fluorescence that needs to be detected is often cut-off as well. Since the number of the optical fiber bundles used for incorporating fluorescence needs to satisfy at least the number of the samples along the beam scanning direction, it is disadvantageous in terms of cost and it requires precise mechanism and adjustment for aligning optical axes.

Furthermore, since the light-receiving angle of the optical fibers is small and thus small amount of fluorescence is incorporated from the sample, an S/N ratio tends to be low. In order to increase the S/N ratio, there has been an attempt to receive only the fluorescence from the sample by using a pulsed laser and acoustooptic modulator (AOM) to attenuate the intensity of the excitation laser beam immediately after exciting the sample. However, pulsed lasers are expensive, and AOM merely attenuates the intensity of the laser beam by 1/1000 and cannot completely prevent the excitation laser beam from contaminating.

#### Disclosure of Invention

The aim of the present invention is to solve the above-described problems concerned with the conventional technique and to provide a method and a device for fluorescence measurement with high detection accuracy with a simple structure by using a relatively inexpensive laser such as a

continuous-wave laser (CW laser) as an excitation light source.

According to the present invention, an excitation light radiating section and a fluorescence detecting section are provided spatially separated from each other. The sample irradiated with excitation light at the excitation light radiating section is transported to the fluorescence detecting section so that the fluorescence can be detected without being interrupted by the excitation light. Accordingly, an S/N ratio is enhanced and thus the above-described aim can be accomplished.

A method for fluorescence measurement of the invention comprises the steps of: irradiating a sample with excitation light from an excitation light source; transporting the sample irradiated with excitation light to an optical axis of a fluorescence detector; and detecting fluorescence incident on the fluorescence detector. The sample comprises a biopolymer labeled with a fluorescent substance.

According to this method, the sample irradiated with excitation light is transported, within a time shorter than the duration time of the fluorescence emitted from the sample, to an optical axis of the fluorescence detector, the optical axis directing towards a position shifted from the excitation light irradiation position. Since the excitation light reflected or scattered off the sample at the excitation light irradiation position is not incident upon the fluorescence

detector, only the fluorescence may be detected with high sensitivity.

A device for fluorescence measurement according to the present invention comprises: an excitation light source; an excitation light radiating means for irradiating a sample with excitation light from the excitation light source; a fluorescence detector for detecting fluorescence emitted from the sample irradiated with excitation light, the fluorescence detector having an optical axis that does not, above the sample, cross with an optical axis of the excitation light radiating means; and a sample transporting means for transporting the sample irradiated with the excitation light of the excitation light radiating means to the optical axis of the fluorescence detector.

In the device for fluorescence measurement, an optical axis of an excitation light radiating means and an optical axis of a fluorescence detector do not cross with each other. The fluorescence of the sample is not measured under the excitation light radiating means but after the sample is transported to the fluorescence detector within a time shorter than the duration time of the fluorescence. By spatially separating the fluorescence irradiation position from the fluorescence detecting position, the excitation light reflected or scattered off the sample at the excitation light irradiation position is not incident upon the fluorescence detector. As a result, only the fluorescence

can be detected with high sensitivity.

Preferably, the fluorescence detector is provided with a photodetector such as PMT and a confocal optical system in which the photodetecting surface of the photodetector and the sample surface are conjugate. The sample transporting means may rotatively transport the sample. A plurality of sets of excitation light sources, excitation light radiating means and fluorescence detectors may be provided for detecting fluorescence of different wavelengths.

The fluorescent substance used with the present invention preferably has long fluorescence duration time and preferably is an Eu (europium) complex such as 4,4'-bis(1",1",1",2",2",3",2"-heptafluoro-4",6"-hexanedion-6"-yl)chlorosulfo-o-terphenyl (BHHCT), rhodamine, FITC, Cy3 and Cy5. For example, BHHCT is a fluorescent substance which emits fluorescence with a wavelength of 615 nm as irradiated with a wavelength of 340 nm, and which has a fluorescent half-life of 100-200  $\mu$ sec which is very long compared to those of conventional fluorescent substances (which are several tens of nsec). By utilizing this property, a high level of fluorescence can be obtained upon irradiating the sample with excitation light and transporting the sample for fluorescence detection, thereby dramatically enhancing an S/N ratio.

#### Brief Description of Drawings

Figure 1 is a schematic view showing an exemplary fluorescence measurement device according to the invention;

Figures 2A and 2B are schematic views for illustrating the positional relationship between an excitation light radiating optical system and a fluorescence detecting optical system;

Figures 3A and 3B are schematic views for illustrating the principle of fluorescence detection according to the invention;

Figure 4 is a schematic view showing another exemplary fluorescence measurement device according to the invention; and

Figure 5 is a schematic view showing a conventional fluorescence measurement device.

#### Best Mode for Carrying Out the Invention

Hereinafter, the present invention will be described in detail with reference to the drawings.

Figure 1 is a schematic view showing an exemplary fluorescence measurement device according to the invention. This device is suitable for reading a circular biochip. The circular biochip 10 is provided with a plurality of sample spots 11 arranged in concentric circles or in a spiral. The biochip 10 is held on a sample platform 12 which may be rotatively driven in a direction represented by an arrow R by a rotating motor 13. Above the biochip 10 is provided an

optical head 20 having an excitation light source 30, an excitation light radiating optical system 40 and a fluorescence detecting optical system 50. For example, on the sample spots 11 which are as microscopic as 50 μm in diameter, a biopolymer such as DNA or a protein labeled with fluorescent substance is hybridized with probe DNAs or the like.

The excitation light source 30 may be, for example, a CW laser. The laser beam emitted from the excitation light source 30 is incident on the excitation light radiating optical system 40, reflected off a mirror 41, converged by an object lens 42 towards the sample spots on the biochip 10. The fluorescence detecting optical system 50 is provided with a light receiving lens 51, a converging lens 52, a fluorescence selection filter 53, a slit 54 and a photomultiplier tube as a photodetector 55, to detect the fluorescence from the sample spots.

The optical head 20 is transported and aligned by a head transporting motor 14 in the radial direction of the biochip 10. When the sample spots 11 are arranged in concentric circles on the biochip 10, the head transporting motor 14 stepwisely aligns the optical head 20 with respect to each concentric circle of the sample spots. When the sample spots 11 are arranged in a spiral on the biochip 10, the head transporting motor 14 continuously transports the optical head 20 to chase the spirally-arranged sample spots

11. A motor controller 15 controls the rotating motor 13 and the head transporting motor 14 to adjustably control the rotational speed of the rotating motor 13 according to the radial position of the optical head 20 on the biochip 10 such that the transporting rate of the sample spots 11 under the optical head 20 is always generally constant.

Figures 2A and 2B are schematic views for illustrating the positional relationship between the excitation light radiating optical system and the fluorescence detecting optical system. Referring to Figure 2A, one of the sample spots 11 on the rotating biochip 10 is irradiated with excitation light from the excitation light source (CW laser) 30 via the excitation light radiating optical system 40 to excite the fluorescent substance contained in the sample spot 11a. Thereafter, as shown in Figure 2B, the sample spot 11a irradiated with excitation light is transported as the biochip 10 rotates to come directly under the fluorescence detecting optical system 50. At this position, the fluorescence emitted from the sample spot 11a is incident on and detected by the fluorescence detecting optical system 50.

Figures 3A and 3B are schematic views for illustrating the principle of the fluorescence detection according to the present invention. Figure 3A shows the state where the sample spot 11a on the transporting biochip 10 is being irradiated with excitation light from the CW laser 30. If the sample spot 11a contains a fluorescence label substance,

the sample spot 11a irradiated with the excitation light will emit fluorescence. The sample spot that has been irradiated with the excitation light will pass under the excitation light radiating optical system 40, and a predetermined time after the irradiation, will come under the fluorescence detecting optical system 50 as shown in Figure 3B. The light receiving lens 51 and the converging lens 52 of the fluorescence detecting optical system 50 form a confocal optical system. The fluorescence emitted from the sample spot 11a scatters in every direction while the fluorescence incident upon the light receiving lens 51 forming the confocal optical system will be converged by the converging lens 52. The converged fluorescence passes through the optical filter 53 and the slit 54 for noise elimination and is incident on the a photodetector (PMT) 55, thereby being detected. Accordingly, fluorescence can be detected without being interrupted by the excitation light. Although the optical axis of the excitation light radiating optical system 40 and the optical axis of the fluorescence detecting optical system 50 are shown parallel to each other in the figure, the optical axes of the two optical systems 40 and 50 may not necessarily be parallel.

Figure 4 is a schematic view for illustrating another example of a fluorescence measurement device of the invention. The fluorescence measurement device is provided with two optical heads 20a and 20b for identifying and detecting

fluorescence from two types of fluorescent substances contained in sample spots. The optical head 20a is provided with an excitation light source (CW laser) 30a, an excitation light radiating optical system 40a and a fluorescence detecting optical system 50a. The optical head 20b is provided with an excitation light source (CW laser) 30b, an excitation light radiating optical system 40b and a fluorescence detecting optical system 50b. The structures of the excitation light radiating optical systems 40a and 40b and the fluorescence detecting optical systems 50a and 50b are identical to those of the excitation light radiating optical system 40 and the fluorescence detecting optical system 50 shown in Figure 1, respectively, and thus description of the details thereof is omitted.

The excitation light sources 30a and 30b are respectively provided with optical filters so that excitation wavelengths can be selected for efficiently exciting different fluorescent substances. The fluorescence detecting optical systems 50a and 50b are respectively provided with optical filters so that wavelength transmitting ranges can be selected for efficiently detecting fluorescence from different fluorescent substances. One optical head 20a radiates excitation light from the excitation light source 30a to a sample spot 11b via the excitation light radiating optical system 40a. After a prescribed time, fluorescence emitted from the sample spot 11b is measured by the

fluorescence detecting optical system 50a without being interrupted by the excitation light, for example, to selectively detect Cy3 contained in the sample spot 11b. The other optical head 20b radiates excitation light from the excitation light source 30b to a sample spot 11c via the excitation light radiating optical system 40b. After a prescribed time, fluorescence emitted from the sample spot 11c is measured by the fluorescence detecting optical system 50b without being interrupted by the excitation light, for example, to selectively detect Cy5 contained in the sample spot 11c.

The two optical heads 20a and 20b are transported by head transporting motors 14a and 14b, respectively, in the radial direction of the biochip 10 to be aligned generally at the same radial distances. When the sample spots 11 are arranged in concentric circles on the biochip 10, the head transporting motors 14a and 14b stepwisely aligns the optical heads 20a and 20b with respect to each concentric circle of the sample spots. When the sample spots 11 are arranged in a spiral on the biochip 10, the head transporting motors 14a and 14b continuously transport the optical heads 20a and 20b to chase the spirally-arranged sample spots 11. A motor controller 15 controls the rotating motor 13 and the head transporting motors 14a and 14b to adjustably control the rotational speed of the rotating motor 13 according to the radial positions of the optical heads 20a and 20b on the

biochip 10 such that the transporting rate of the sample spots 11 under the optical heads 20a and 20b is almost generally constant.

#### Industrial Applicability

According to the present invention, fluorescence from a fluorescent substance contained in a sample spot may be measured with high sensitivity without being interrupted by excitation light.

## CLAIMS

1. A method for measuring fluorescence comprising the steps of:

irradiating a sample with excitation light from an excitation light source;

transporting the sample irradiated with excitation light to an optical axis of a fluorescence detector; and

detecting fluorescence incident on the fluorescence detector.

2. A method for measuring fluorescence according to claim 1, wherein the sample comprises a biopolymer labeled with a fluorescent substance.

3. A fluorescence measurement device comprising:

an excitation light source;

an excitation light radiating means for irradiating a sample with excitation light from the excitation light source;

a fluorescence detector for detecting fluorescence emitted from the sample irradiated with excitation light, the fluorescence detector having an optical axis that does not, above the sample, cross with an optical axis of the excitation light radiating means; and

a sample transporting means for transporting the sample

irradiated with the excitation light of the excitation light radiating means to the optical axis of the fluorescence detector.

4. A fluorescence measurement device according to claim 3, wherein the sample transporting means rotatively transports the sample and wherein the device is provided with a plurality of sets of excitation light sources, excitation light radiating means and fluorescence detectors for detecting fluorescence of different wavelengths.

FIG. 1

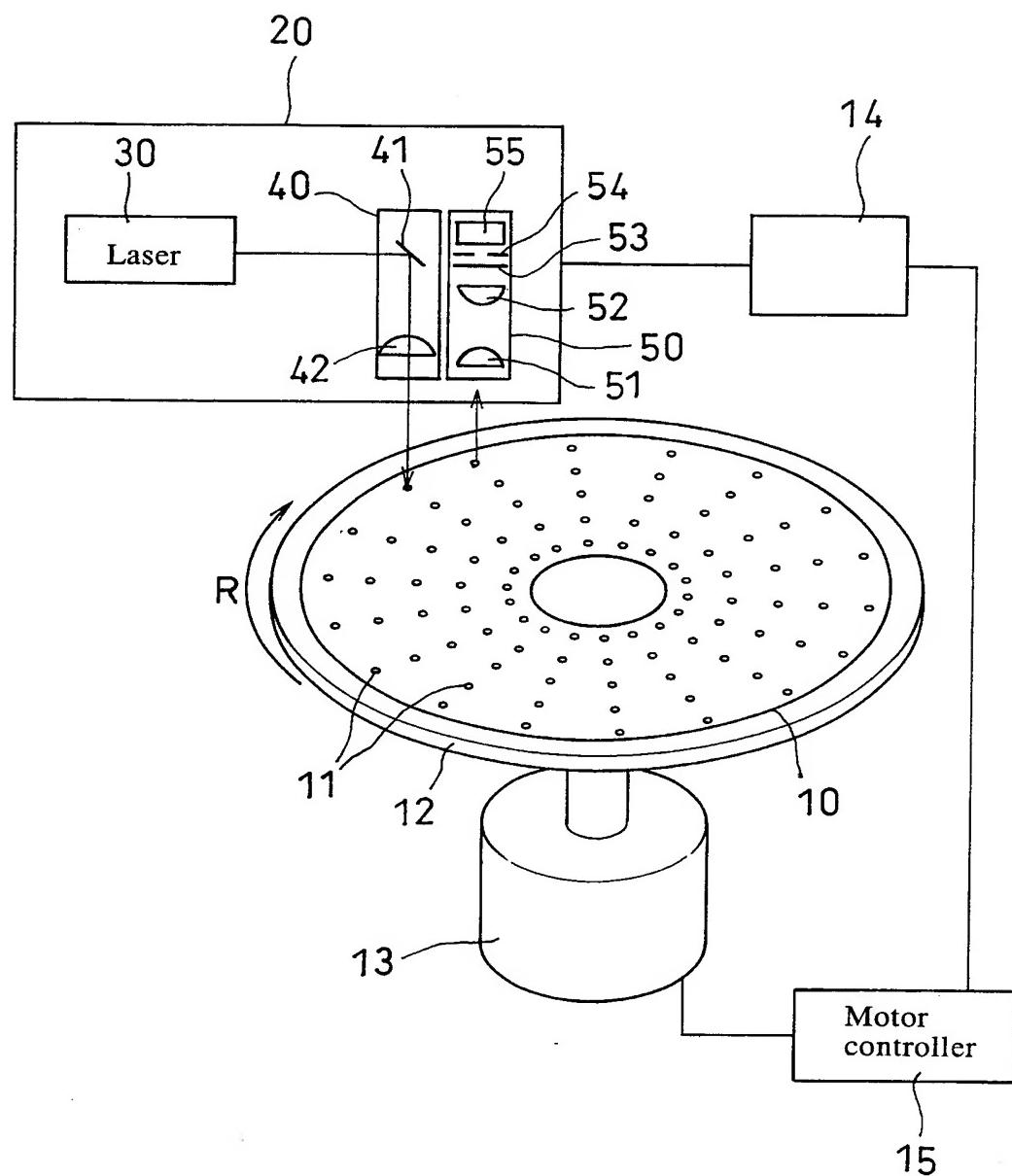


FIG. 2A

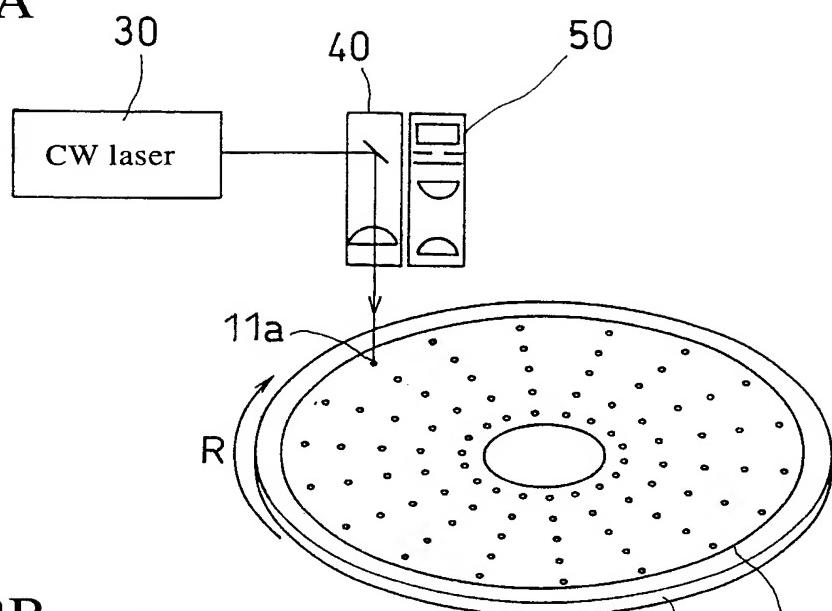
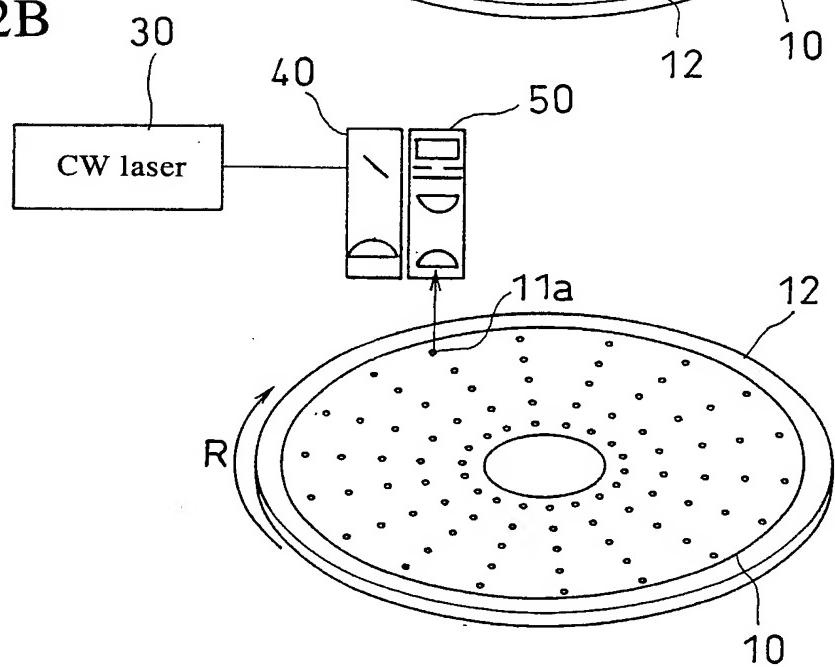


FIG. 2B



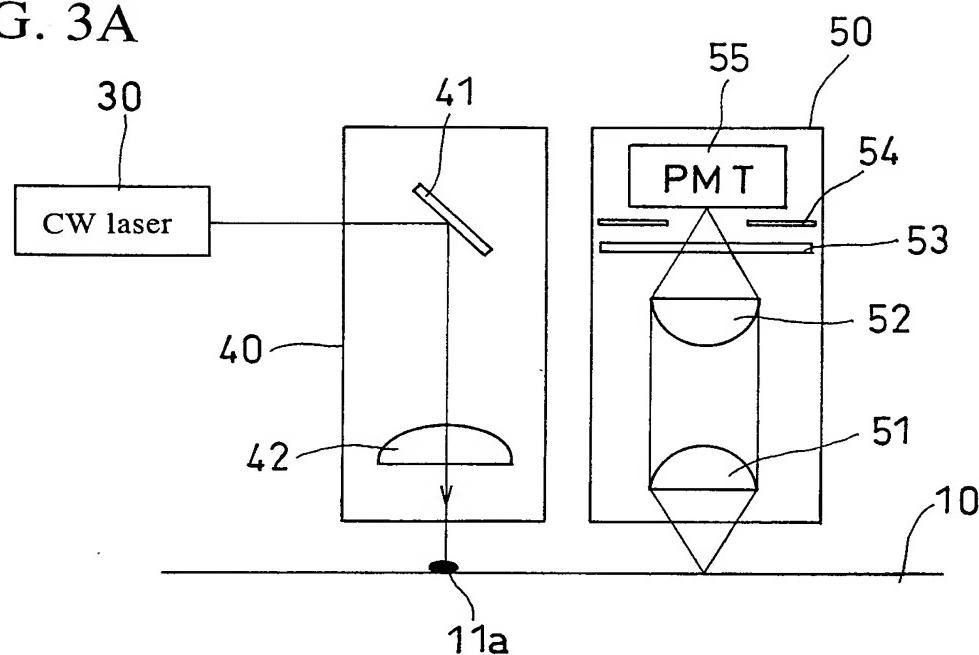
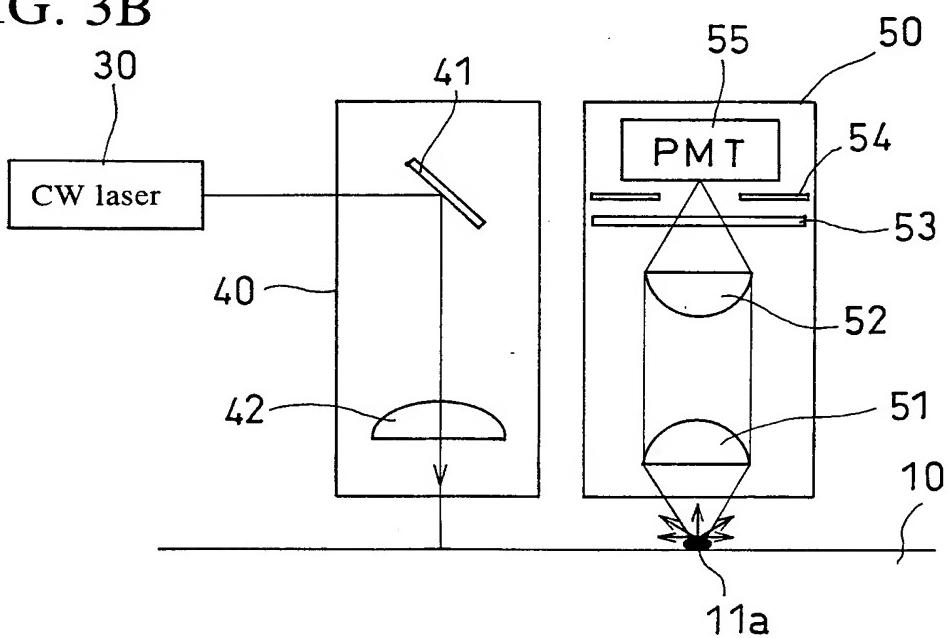
**FIG. 3A****FIG. 3B**

FIG. 4

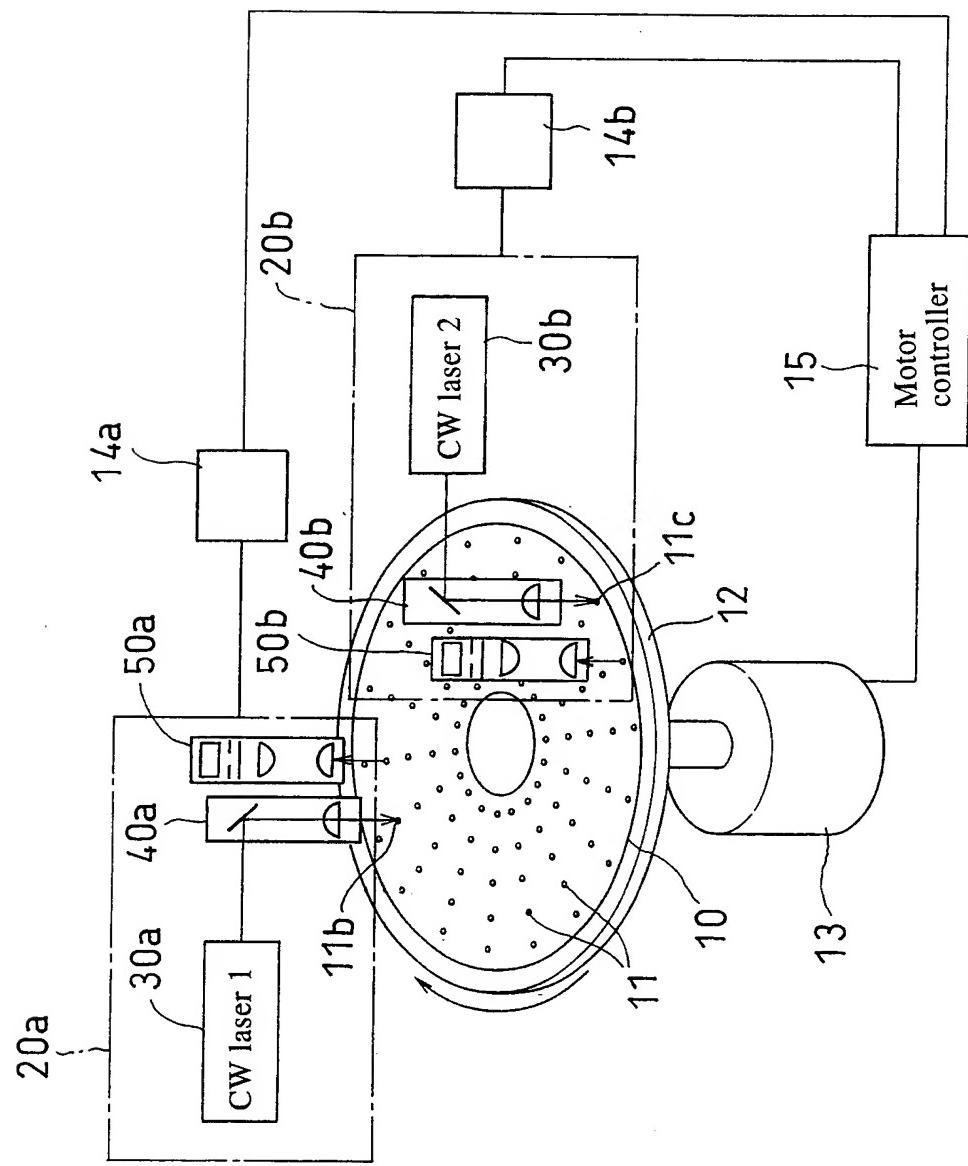
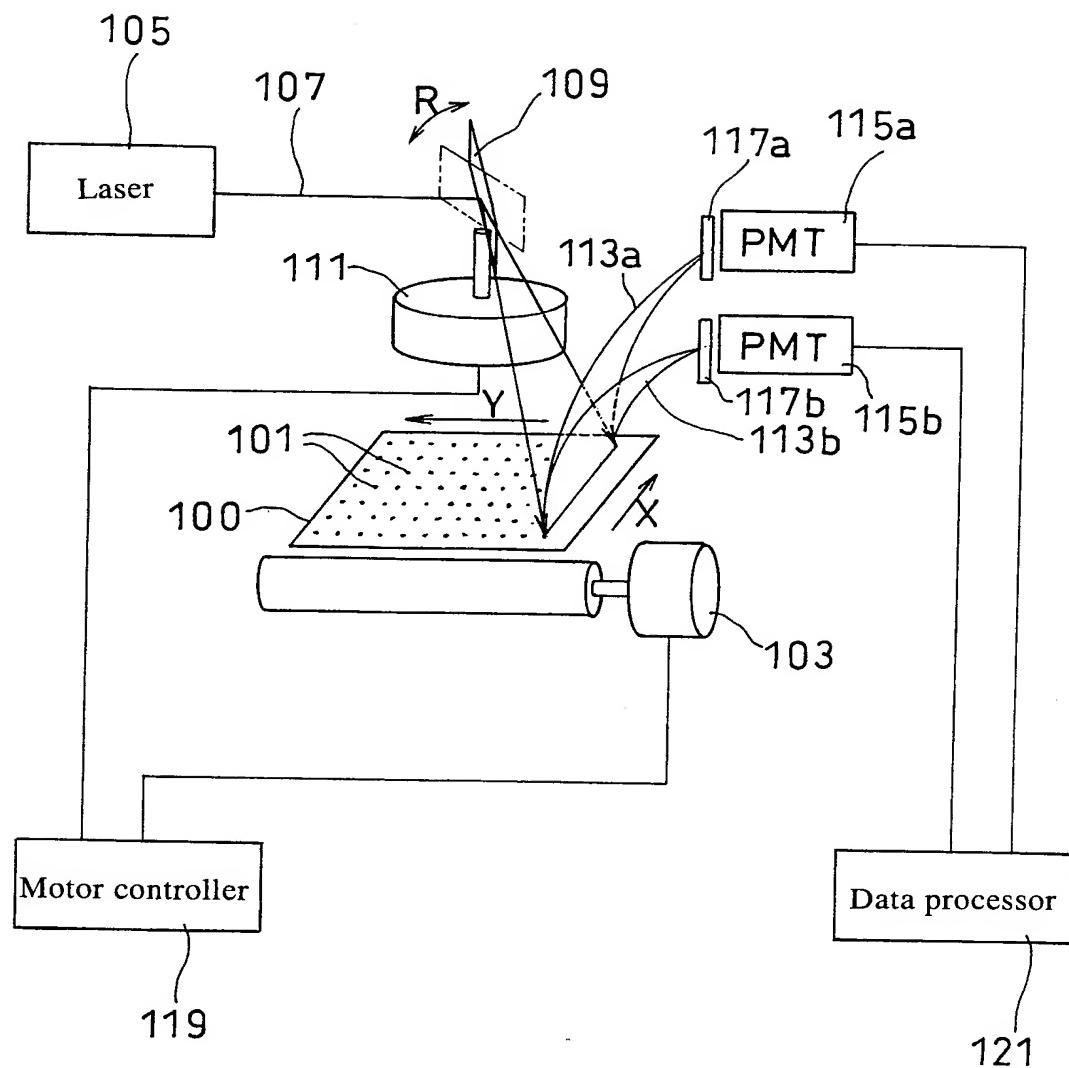


FIG. 5



## INTERNATIONAL SEARCH REPORT

International Application No  
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A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 G01N21/64 G01N33/487

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 778 763 A (MAKIGUCHI KYOKO ET AL) 18 October 1988 (1988-10-18)	1,3
Y	abstract column 4, line 19 - line 66 figure 1 —	2,4
Y	WO 94 02645 A (RES DEV FOUNDATION) 3 February 1994 (1994-02-03) claims 1,38 —	2
Y	DE 197 07 226 A (BODENSEEWERK PERKIN ELMER CO) 27 August 1998 (1998-08-27) column 5, line 29 - line 53 figures 1,2 —	4
A	JP 11 094747 A (HITACHI SOFTWARE ENG CO LTD) 9 April 1999 (1999-04-09) abstract —	1

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Patent family members are listed in annex.

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/JP 00/02996

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
US 4778763 A	18-10-1988	JP	1632865	C	26-12-1991
		JP	2055744	B	28-11-1990
		JP	61241639	A	27-10-1986
		DE	3673681	D	04-10-1990
		EP	0198513	A	22-10-1986
WO 9402645 A	03-02-1994	AU	4775293	A	14-02-1994
		CN	1083113	A	02-03-1994
DE 19707226 A	27-08-1998	AU	5753998	A	18-09-1998
		WO	9838495	A	03-09-1998
		EP	0961929	A	08-12-1999
JP 11094747 A	09-04-1999	NONE			